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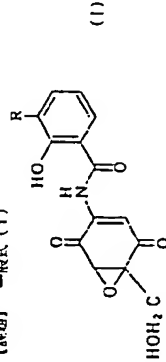
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(54)【発明の名称】 新規抗菌物質エポキシノマイシンAおよびBとその製造法

(57)【要約】

【課題】 メキシリン耐性菌を含むグラム陰性菌に対する抗菌活性および抗腫瘍活性を示す新しい分子骨格を有する抗菌物質を提供する。

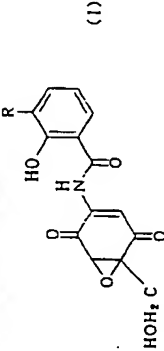
【解決】 一般式(1)



(式中、RはエポキシノマイシンAでは塩基原子を示し、エポキシノマイシンBでは水素原子を示す)で表わされるエポキシノマイシンAおよびエポキシノマイシンBが新規抗菌物質としてアミコラトブシス sp. WZ299-95F4株の培養により得られた。エポキシノマイシンAおよびB、あるいはそれらの塩は各種金属との塩、例えばナトリウムのようなアルカリ金属との塩があり、これらの塩も上記の抗菌活性と抗腫瘍活性を有する。

【特許請求の範囲】

【請求項1】 次的一般式(1)：



(式中、RはエポキシノマイシンAでは塩基原子を示し、またエポキシノマイシンBでは水素原子を示す)で表わされる化合物である抗菌物質エポキシノマイシンAおよびエポキシノマイシンB、またはそれらの塩。

【請求項2】 アミコラトブシス菌に属する、請求項1に記載のエポキシノマイシンAおよびBの生産菌を炭素培地に培養し、培養物からエポキシノマイシンAおよび(または)Bを採取することを特徴とする、抗菌物質エポキシノマイシンAおよび(または)エポキシノマイシンBの製造法。

【請求項3】 抗菌物質エポキシノマイシンAおよび(または)エポキシノマイシンB、またはそれらの塩を有効成分とする抗腫瘍剤。

【請求項4】 抗菌物質エポキシノマイシンAおよび(または)エポキシノマイシンB、またはそれらの塩を有効成分とする抗腫瘍剤。

【請求項5】 抗菌物質エポキシノマイシンAおよびエポキシノマイシンBを生産する特性を持つアミコラトブシス sp. WZ299-95F4株。

【発明の詳細な説明】

【0001】
【発明の属する技術分野】 本発明は、抗菌活性及び抗腫瘍活性または抗腫瘍活性を示す新規抗菌物質エポキシノマイシン(Epoxyquinolone) A およびエポキシノマイシンB、あるいはこれらとの塩に、またエポキシノマイシンAおよび(または)エポキシノマイシンBの製造法に関する。さらに本発明は、エポキシノマイシンAおよび(または)エポキシノマイシンBまたはそれらの塩を有効成分とする抗腫瘍剤及び抗腫瘍剤に関する。また、本発明は新規抗菌物質エポキシノマイシンAおよびBを生産する特性を持つ新規微生物としてのアミコラトブシス sp. WZ299-95F4株を含む。

【0002】
【従来の技術】 種々な多数の抗菌物質が知られており、また種々な多数の抗腫瘍物質が知られている。

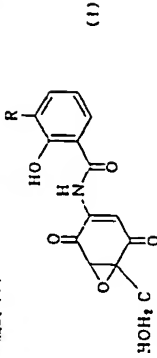
【0003】
【発明が解決しようとする課題】 細菌感染症の化学療法において、多剤耐性菌の出現は重大な問題である。従来知られているまたは使用されている既知の抗菌性化合物

とは、異なる化学構造を有し且つ優れた抗菌活性を示す新しい化合物の発見または創製をすることは常に望まれており、そのための研究が行われている。また抗腫瘍性物質は、一般に強い毒性を有するものが多く、その副作用としての使用に当たって大きな制約となっている。そこで、毒性が低く且つ新規な化学構造を有する抗腫瘍性物質を見出すことは創製することが常に望まれており、そのための研究が行われている。

【0004】

【課題を解決するための手段】 本発明者らは、上記の要望に応えることができる抗菌活性及び抗腫瘍活性を有する新規な抗菌物質を提供することを目的に、従来より有用な抗菌物質の構造と類似した化合物の合成を試み、その結果、土壌細菌から新規な微生物としてアミコラトブシス菌に属する菌株を分離することに成功し、またこの菌株が新しい抗腫瘍特性を有する種々の抗菌物質を生産していることを見出した。これら新規抗菌物質を調製すること成功し、それぞれにエポキシノマイシンAおよびエポキシノマイシンBと命名した。更に、これら新規抗菌物質が薬剤耐性菌(メキシリン耐性菌等)をよく含むグラム陰性の細菌に抗菌活性を示し、また癌細胞の増殖に対して抑制活性を示すことを見出した。

【0005】すなわち、第1の本発明においては、次の一般式(1)：



(式中、RはエポキシノマイシンAでは塩基原子を示し、またエポキシノマイシンBでは水素原子を示す)で表わされる化合物であるエポキシノマイシンAおよびエポキシノマイシンB、あるいはこれらとの塩が提供される。

【0006】エポキシノマイシンAおよびBは、耐性菌であり、それらの塩としては、第4段アミン、銅、鉛などの有機塩基との塩、あるいは各種金属との塩、例えばナトリウムのようなアルカリ金属との塩があり、これらの塩も上記の抗菌活性と抗腫瘍活性を有する。

【0007】次に、抗菌物質エポキシノマイシンAおよびBの物理化学的性質を記載する。

(1) エポキシノマイシンAの物理化学的性質

A) 外観及び性質：淡黄色粉末、結晶性物質

B) 融点：168-173°C (分解)

C) 比旋光度：[α]_D²⁵ +44.6° (c 0.51, メタノール)

D) T.L.CのR_f値：0.28

シリカゲル (Art. 105715、マルク社製) の薄層クロマト

グラフィーで展開溶媒としてクロロホルム-メタノール

E) マススペクトル (m/z) : 324, 326 (M+H)・

322, 324 (M-H)・

F) 高分解能マススペクトル : 質量値 322.0136 (M-H)・

計算値 322.0118

G) 分子式 : $C_{14}H_{16}NO_4$ C 1 H 1 酸素吸収スペクトル :

(1) メタノール溶液中で測定したUV吸収スペクトル

は添付図面の図1に示す。主なピークは次のとおりであ

る。

J) ^{13}C -NMRスペクトル (CD₃OD/TMS) : 添

付図面の図5に示す。

K) 1H -NMRスペクトル (CD₃OD/TMS) : 添

付図面の図6に示す。

【0008】(2) エボキシノマイシンBの理化学的性

状

A) 外観及び性質 : 淡黄色粉末、揮発性物質

B) 融点 : 178-184°C (分解)

C) 比吸収度 : (c) \times d + 32.2° (c 0.51, メタノ

ール)

D) TLCのR_f値 : 0.52

シリカゲル (Art. 105715, メルク社製) の薄層クロマト

グラフィーで展開溶媒としてクロロホルム-メタノール

(10:1) で展開して測定した場合

E) マススペクトル (m/z) : 280 (M)・

288 (M-H)・

F) 高分解能マススペクトル : 質量値 290.0656 (M+H)・

計算値 290.0664

G) 分子式 : $C_{14}H_{16}NO_4$

H) 紫外吸収スペクトル :

(1) メタノール溶液中で測定したUV吸収スペクトル

は添付図面の図7に示す。主なピークは次のとおりであ

る。

J) ^{13}C -NMRスペクトル (CD₃OD/TMS) : 添

付図面の図11に示す。

K) 1H -NMRスペクトル (CD₃OD/TMS) : 添

付図面の図12に示す。

【0009】さらに、抗生物質エボキシノマイシンA

およびBの生物学的性状を次に記載する。

【0010】A) 抗悪性

本発明による抗生物質エボキシノマイシンAおよびB

の胃腸炎菌毒素に対する殺菌作用を抑制する殺菌作用阻

断度は、次の表1に示す通りである。この抗悪性ベク

トルは日本化学療法学会標準法に基づき、ミューリン

トン毒素で菌数増殖法により測定した。

【0011】

1) 紫外吸収スペクトル (KBr錠剤法) : 添付図面

λ_{max} nm (c) 252(5700), 327(6500)

2) 紫外吸収スペクトル (KBr錠剤法) : 添付図面

λ_{max} nm (c) 252(5700), 327(6500)

3) 紫外吸収スペクトル (KBr錠剤法) : 添付図面

λ_{max} nm (c) 252(5700), 327(6500)

4) 紫外吸収スペクトル (KBr錠剤法) : 添付図面

λ_{max} nm (c) 252(5700), 327(6500)

5) 紫外吸収スペクトル (KBr錠剤法) : 添付図面

λ_{max} nm (c) 252(5700), 327(6500)

6) 紫外吸収スペクトル (KBr錠剤法) : 添付図面

λ_{max} nm (c) 252(5700), 327(6500)

7) 紫外吸収スペクトル (KBr錠剤法) : 添付図面

λ_{max} nm (c) 252(5700), 327(6500)

8) 紫外吸収スペクトル (KBr錠剤法) : 添付図面

λ_{max} nm (c) 252(5700), 327(6500)

9) 紫外吸収スペクトル (KBr錠剤法) : 添付図面

λ_{max} nm (c) 252(5700), 327(6500)

(表1)

試 験 値	最低発育阻害濃度 (μ g/ml)	
	エボキシノマイシンA	エボキシノマイシンB
スタヒロコカス・アウレウス FIM 209P	12.5	12.5
スタヒロコカス・アウレウス・スミス	12.5	12.5
スタヒロコカス・アウレウス MS 9610	50	25
スタヒロコカス・アウレウス WISA No.5	25	25
スタヒロコカス・アウレウス MS 16528	25	25
スタヒロコカス・アウレウス TY-04282	50	25
スタヒロコカス・アウレウス FIM 16	12.5	25
ミクロコッカス・ルテウス IFD 3330	3.12	0.25
バシラス・アンスラシス	25	12.5
バシラス・サブチリス NRRL 8-558	50	12.5
バシラス・セウス ATCC 10702	25	12.5
コリネバクテリウム・ホビス 1810	50	50
エシェリヒア・コリ NIHJ	100	50
エシェリヒア・コリ BE 1121	50	50
エシェリヒア・コリ B6 1186	50	50
シガラ・ディセンテリエ JS 11910	50	50
シウトモナス・エルギノサ A.3	>50	>50
バクステラ・ヒンシダ sp. 8395	12.5	12.5
バクステラ・ヒンシダ sp. 0356	12.5	12.5
バクステラ・ヒンシダ p-3347	3.12	12.5

【0012】B) 癌細胞増殖抑制活性

各種の癌細胞を用いて癌細胞の増殖を50%抑制するエボ

キシノマイシンAおよびエボキシノマイシンBの濃

度 (IC₅₀ 値) を、MTT法 (「Journal of Immunologic

第2)

供 試 癌 細 胞	IC ₅₀ (μ g/ml)	
	エボキシノマイシンA	エボキシノマイシンB
マウス白血病 L1210	2.64	16.3
マウス IMCカルシノーマ	9.67	17.9
マウスサルコーマ S180	7.67	
マウス黒色腫 B16-B1.6	7.97	

【0014】表1の結果から明らかなように、本発明に

よる抗生物質エボキシノマイシンAおよびBは、各種

の細胞に対して抗悪性活性を有するから抗悪性として有用

である。また、表2の結果から明らかなように、エボキ

シノマイシンAおよびBは、各種の癌細胞の増殖を抑制

する抗腫瘍活性または抗悪性活性を有するから抗腫瘍利

用である。また、表2の結果から明らかなように、エボキ

シノマイシンAおよびBは、各種の癌細胞の増殖を抑制

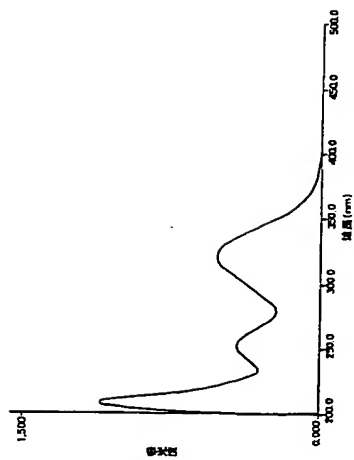
する抗腫瘍活性または抗悪性活性を有するから抗腫瘍利

用である。また、表2の結果から明らかなように、エボキ

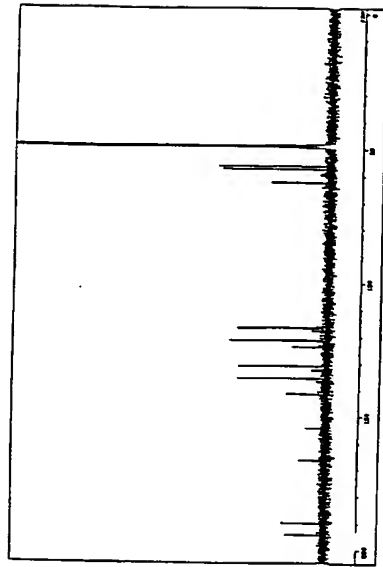
シノマイシンAおよびBは、各種の癌細胞の増殖を抑制

する抗腫瘍活性または抗悪性活性を有するから抗腫瘍利

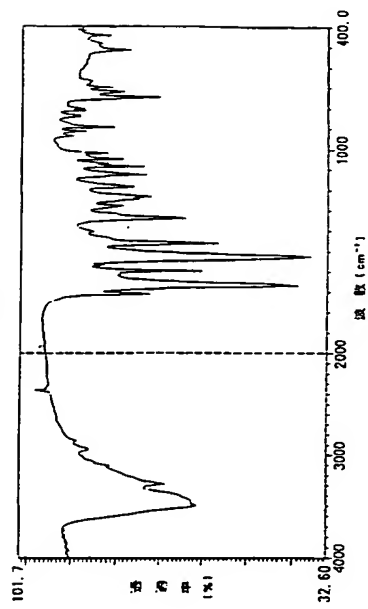
【図3】



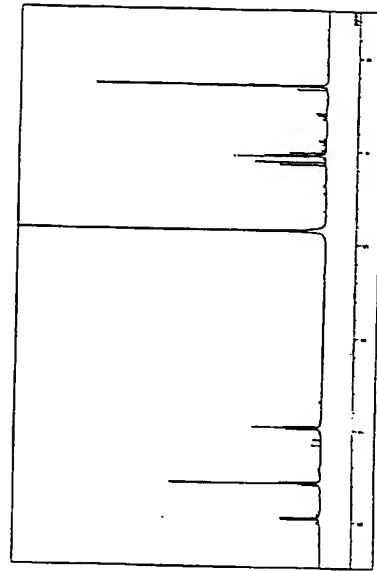
【図5】



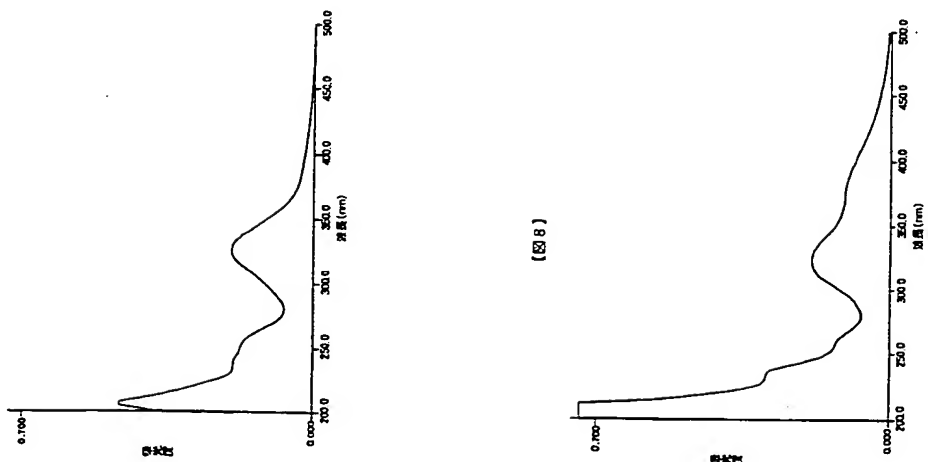
【図4】



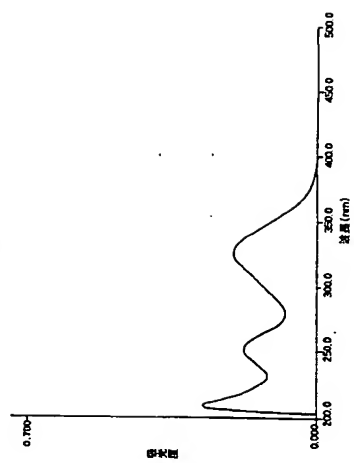
【図6】



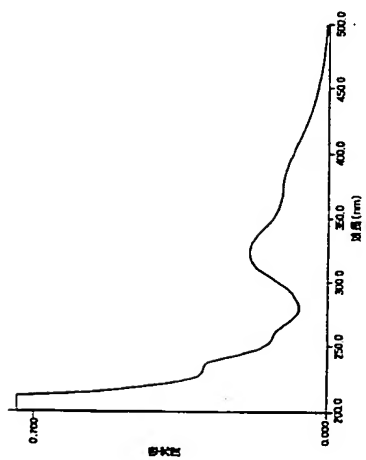
【図 7】



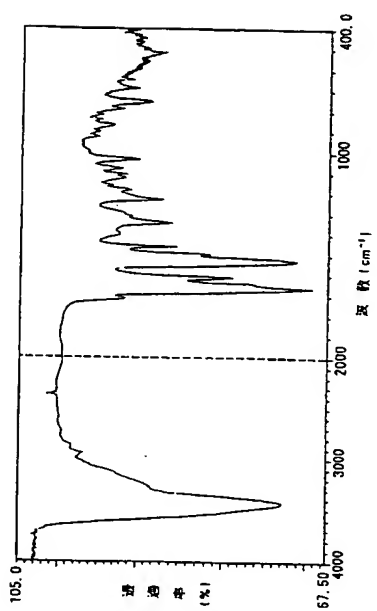
【図 9】



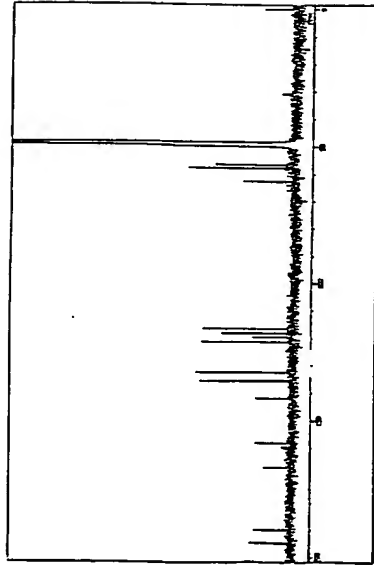
【図 8】



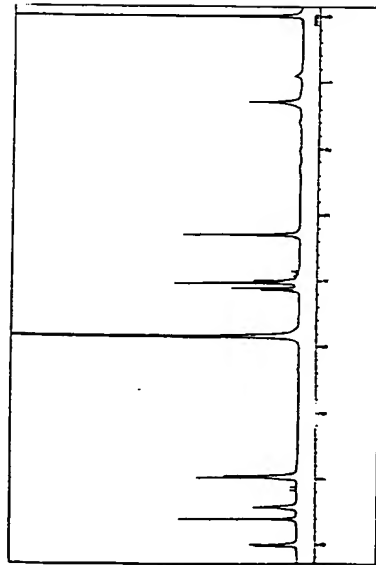
【図 10】



【図11】



【図12】



【手続補正書】
【提出日】平成8年4月26日
【手続補正1】
【補正対象書類名】明細書
【補正対象項目名】0010
【補正方法】変更
【補正内容】
【0010】A) 抗菌活性

本発明による抗生物質エポキシノマイシンAおよびBの各種細菌に対する最低発育阻止濃度は、次の表1に示す通りである。この抗菌スペクトルは日本化学療法学会標準法に基き、ミューラー-ヒントントン寒天培地で倍數希釈法により測定した。
【手続補正2】
【補正対象書類名】明細書

【補正対象項目名】0021

【補正方法】変更

【補正内容】

【0021】(4) 炭素源の利用性（ブリードハム・ゴドリーフ寒天培地、ISP-培地9：27℃培養）
d-グルコース、d-フルクトース、イノシトール、d-マンニトールを利用して発育し、L-アラビノース、シュクロース、ラムノース、ラフィノースは利用しない。

フロントページの続き

(51)Int. Cl.⁴
C12R 1:01

F I

技術表示箇所

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い、d-キシロースの利用の存在は利便としない。
(5) リンゴ酸石灰の溶解（リンゴ酸石灰寒天培地、27℃培養）
培養後10日目頃よりリンゴ酸石灰の溶解が認められ、その作用は中等度である。
(6) 固着性の還元反応（0.1%固着カリウムを含むペプトン水、ISP-培地8、27℃培養）
陰性である。

NOVEL ANTIBIOTICS EPOXYQUINOMICINS A AND B,
AND METHOD FOR PRODUCING THE SAME

__ABSTRACT

PROBLEM

Antibiotics with new molecular skeletons that exhibit antibacterial activity and antitumor activity against Gram-positive bacteria including methicillin resistant *Staphylococcus aureus* (MRSA) are provided.

PROBLEM

Epoxyquinomicin A and epoxyquinomicin B, represented by the general formula (I), were obtained as novel antibiotics by culturing *Amycolatopsis* sp. MK299-95F4.

(wherein R represents chlorine and hydrogen in epoxyquinomicin A and epoxyquinomicin B, respectively) Epoxyquinomicins A and B or their salts are antibiotics with antibacterial activity and antitumor activity against various bacteria.

CLAIMS

1. Epoxyquinomicin A and epoxyquinomicin B, which are compounds represented by the following general formula (I) or their salts,

(wherein R represents chlorine and hydrogen in epoxyquinomicin A and epoxyquinomicin B, respectively).

2. A method for producing the antibiotics epoxyquinomicins A and (or) B, comprising the steps of culturing in a nutrient medium a bacterium of the genus *Amycolatopsis*, which produces epoxyquinomicins A and B of claim 1, and isolating epoxyquinomicins A and (or) B from the resulting culture.

3. An antibacterial agent, comprising epoxyquinomicin A and (or) epoxyquinomicin B or their salt(s) as active

ingredient(s).

4. An antitumor agent, comprising epoxyquinomicin A and (or) epoxyquinomicin B or their salt(s) as active ingredient(s).

5. Amycolatopsis sp. MK299-95F4, which has the property of producing the antibiotics epoxyquinomicin A and epoxyquinomicin B.

BRIEF SUMMARY OF THE INVENTION

[0001]

TECHNICAL FIELD

The present invention relates to novel antibiotics epoxyquinomicin A and epoxyquinomicin B with antibacterial activity and antitumor/anticancer activity or their salts, and also to a method for producing epoxyquinomicins A and (or) epoxyquinomicin B. The present invention further relates to antibacterial agents and antitumor agents which contain epoxyquinomicin A and (or) epoxyquinomicin B or their salt as active ingredient(s). In addition, the present invention encompasses Amycolatopsis sp. MK299-95F4, which has the property of producing the antibiotics epoxyquinomicin A and epoxyquinomicin B.

[0002]

DESCRIPTION OF THE RELATED ART

Large numbers of various antibacterial agents and large numbers of various antitumor substances are known.

[0003]

PROBLEM TO BE SOLVED BY THE INVENTION

The emergence of multidrug-resistant bacteria is a major concern in the chemotherapy of microbism. Discovery and development of new compounds that have different chemical structures from known antibacterial compounds currently used or conventionally known and that exhibit excellent antibacterial activity have always been awaited and thus studies aimed at that scope has been promoted. In general, most antitumor substances have strong toxicity, which poses serious limitations to their use as antitumor agents. Discovery and

development of new compounds that are less toxic with novel chemical structures have always been awaited and thus studies aimed at that scope has been promoted.

[0004]

MEANS FOR SOLVING THE PROBLEMS

The inventors have long promoted the research and development of useful antibiotics and their practical use for the purpose of providing novel antibiotics with the antibacterial activity and antitumor activity that can meet the aforementioned request. As a result, we have succeed in isolating a strain of the genus Amycolatopsis as a novel microorganism from soil samples and found the strain produces a plurality of antibiotics with new structural skeletons. We have successfully isolated two new types of novel antibiotics and named each epoxyquinomicin A and epoxyquinomicin B. Further, we have found that these novel antibiotics exhibit antibacterial activity against Gram-positive bacteria including drug-resistant bacteria (methicillin resistant Staphylococcus aureus etc.) and suppressive activity on proliferation of cancer cells.

[0005] Accordingly, in a first aspect of the present invention, there is provided epoxyquinomicin A and epoxyquinomicin B, which are compounds represented by the following general formula (I) or their salts,

(wherein R represents chlorine and hydrogen in epoxyquinomicin A and epoxyquinomicin B, respectively)

[0006] Epoxyquinomicins A and B are acescent substances. Their salts include salts with organic bases, such as quaternary ammonium salt, or salts with various metals, and salts with alkali metals, such as sodium. These salts also have the aforementioned antibacterial activity and antitumor activity.

[0007] Hereinbelow, the physical and chemical properties of the antibiotic epoxyquinomicins A is described.

(1) The physical and chemical properties of the antibiotic epoxyquinomicins A

A) Appearance and properties: light yellow powder, acescent substance

B) Melting point: 168-173°C (degradation)

C) Specific rotation: $[\alpha]_D^{25} + 44.6^\circ$ (c 0.51, methanol)

D) Rf value in TLC: 0.28

When developed on silica gel-coated thin layer chromatography plates. (Merck Art 105715) using chloroform/MeOH = 10:1 as a developing solvent

E) Mass spectrum (m/z): 324, 326 (M+H)⁺
322, 324 (M-H)⁻

F) High resolution mass spectrum:

Experimental value 322.0136 (M-H)⁻

Calculated value 322.0118

G) Molecular formula: C₁₄H₁₀NO₆ Cl

H) Ultraviolet absorption spectra:

(i) UV absorption spectrum measured in methanol solution is shown in FIG. 1 of the accompanying drawing. The characteristic peaks are as follows:

λ max nm (ϵ) 236 (sh, 8900), 255 (sh, 5900), 325 (8000), 370 (sh, 2700)

(ii) UV absorption spectrum measured in 0.01N NaOH-methanol solution is shown in FIG. 2 of the accompanying drawing. The following characteristic peaks were observed:

λ max nm (ϵ) 234 (sh, 11600), 257 (sh, 5100), 327 (8300), 371 (sh, 4400)

(iii) UV absorption spectrum measured in 0.01N NCl-methanol solution is shown in FIG. 3 of the accompanying drawing. The following characteristic peaks were observed:

λ max nm (ϵ) 253 (6700), 322 (8500)

I) Infrared absorption spectrum (the KBr tablet method): shown in FIG. 4 of the accompanying drawing.

ν max (cm⁻¹) 3450, 1710, 1670, 1600, 1520, 1460, 1340, 1230

J) ¹³C-NMR spectrum (CD₃OD/TMS): shown in FIG. 5 of the accompanying drawing.

K) ¹H-NMR spectrum (CD₃OD/TMS): shown in FIG. 6 of the

accompanying drawing.

[0008] (2) The physical and chemical properties of the antibiotic epoxyquinomicins B

A) Appearance and properties: light yellow powder, acescent substance

B) Melting point: 178-184°C (degradation)

C) Specific rotation: $[\alpha]_D^{25} + 32.2$ (c 0.51, methanol)

D) Rf value in TLC: 0.52

When developed on silica gel-coated thin layer chromatography plates (Merck Art 105715) using chloroform/MeOH = 10:1 as a developing solvent

E) Mass spectrum (m/z): 289 (M)⁺
288 (M-H)⁻

F) High-resolution mass spectrum:

Experimental value 290.0656 (M+H)⁺

Calculated value 290.0664

G) Molecular formula: C₁₄H₁₁NO₆

H) Ultraviolet absorption spectra:

(i) UV absorption spectrum measured in methanol solution is shown in FIG. 7 of the accompanying drawing. The following characteristic peaks can be observed:

λ max nm (ϵ) 237(6100), 253(sh, 5400), 326(6300),

(ii) UV absorption spectrum measured in 0.01N NaOH-methanol solution is shown in FIG. 8 of the accompanying drawing. The following characteristic peaks were observed:

λ max nm (ϵ) 235 (9100), 259 (sh, 4000), 327 (5800), 376 (sh, 3400)

(iii) UV spectrum measured in 0.01N NCl-methanol solution is shown in FIG. 9 of the accompanying drawing. The following characteristic peaks were observed:

λ max nm (ϵ) 252(5700), 327(6500)

I) Infrared absorption spectrum (the KBr tablet method): shown in FIG. 10 of the accompanying drawing.

ν max (cm⁻¹) 3430, 1710, 1660, 1610, 1530, 1340, 1230

J) ^{13}C -NMR spectrum ($\text{CD}_3\text{OD/TMS}$): shown in FIG. 11 of the accompanying drawing.

K) ^1H -NMR spectrum ($\text{CD}_3\text{OD/TMS}$): shown in FIG. 12 of the accompanying drawing.

[0009] Further, the biological properties of the antibiotics epoxyquinomicins A and B are described below.

[0010] A) Antibacterial activity

The minimum growth inhibition concentrations of the antibiotics epoxyquinomicins A and B according to the present invention against various bacteria on a normal nutrient agar plate is as shown in Table 1 below. This antibacterial spectrum was obtained by the two-fold dilution method by the Muller-Hinton agar medium according to the standard method recommended by Japanese Society of Chemotherapy.

[0011]

Test microorganisms	Minimum growth inhibition concentration ($\mu\text{g/ml}$)	
	epoxyquinomicin A	epoxyquinomicin B
Staphylococcus aureus FDA 209P	12.5	12.5
Staphylococcus aureus Smith	12.5	12.5
Staphylococcus aureus MS 9610	50	25
Staphylococcus aureus MRSA No.5	25	25
Staphylococcus aureus MS 16526	25	25
Staphylococcus aureus TY-04282	50	25
Micrococcus luteus FDA-16	12.5	25
Micrococcus luteus IFO 3333	3.12	6.25
Bacillus anthracis	25	12.5
Bacillus subtilis NRRL B-558	50	12.5
Bacillus cereus ATCC 10702	25	12.5
Corynebacterium bovis 1810	50	50
Escherichia coli NIHJ	100	50
Escherichia coli BE 1121	50	50
Escherichia coli BE 1186	50	50
Shigella dysenteriae JS 11910	50	50
Pseudomonas aeruginosa A3	>50	>50
Pasteurella piscicida sp. 6395	12.5	12.5
Pasteurella piscicida sp. 6356	12.5	12.5
Pasteurella piscicida p-3347	3.12	12.5

[0012] B) Suppressive activity on cancer cell proliferation
The concentrations of epoxyquinomicin A and epoxyquinomicin B that cause 50% cancer cell death (IC_{50}) were determined using various kinds of cancer cells by the MTT method (refer to "Journal of Immunological Methods," 65, 55-60 (1983)). The results are shown in Table 2.

[0013] Table 2

Test cancer cells	IC ₅₀ (μg/ml)	
	epoxyquinomicin A	epoxyquinomicin B
Mouse leukemia L1200	2.64	16.3
Murine carcinoma IMC carcinoma	9.67	17.9
Murine sarcoma S180	7.67	
Mouse melanoma B16-BL6	7.97	

[0014] As is apparent from the results in Table 1, since the antibiotics epoxyquinomicins A and B according to the present invention have antibacterial activity against various kinds of bacteria, they are useful as antimicrobial agents. Further, as is apparent from the results in Table 2, since epoxyquinomicins A and B have antitumor activity or anti-cancer activity in which proliferation of various kinds of cancer cells are suppressed, they are useful as antitumor agents or anticancer agents.

[0015] Further, according to a second aspect of the present invention, a method is provided for producing the antibiotics epoxyquinomicin A and (or) B, which include the steps of culturing in a nutrient medium a bacterium of the genus *Amycolatopsis*, which produces epoxyquinomicins A and B of claim 1, and isolating epoxyquinomicins A and (or) B from the resulting culture.

[0016] One example of the epoxyquinomicins A and B-producing bacteria that can be used in a second aspect of the present invention is *Amycolatopsis* sp. MK299-95F4. This is an actinomycetes strain designed as MK299-95F4, which was isolated by the present inventors from a soil sample collected in Sendai city, Miyagi prefecture, Japan, in October, 1994.

[0017] The microbial properties of MK299-95F4 are as follows:

1. Morphological observation

Substrate hyphae are extensively branched, giving a zigzag appearance. Fragmentation is observed. Aerial hyphae are either straight or irregularly curving, disintegrating into oval- to cylindrical-structures or spore-like structure.

Spores measure about 0.4 to 0.6 μ by 1.1 to 1.6 μ in size and have a smooth surface. Neither verticillate branching nor rhizomorph nor sporangium nor motile spore is observed.

[0018] 2. Growth characteristics in various culture media

The standard given in each of the brackets for the description of color is according to "Color Harmony Manual" of Container Corporation of America.

(1) Sucrose-nitrate-agar culture medium (cultured at 27°C)
White aerial hyphae are thinly formed on the colorless growth. No soluble pigment is observed.

(2) Glucose-asparagine-agar medium (cultured at 27°C)
White aerial hyphae are formed on pale yellow (2 ea, Lt Wheat-2 gc Bamboo) growth. The soluble pigment is tinged with yellow.

(3) Glycerine-asparagine-agar medium (ISP-medium 5, cultured at 27°C)

White aerial hyphae are formed on yellowish brown [3 ie, Camel-31e, and Cinnamon] growth. The soluble pigment is tinged with yellowish brown.

(4) Starch-inorganic salt-agar medium (ISP-medium 4, cultured at 27°C)

White aerial hyphae are thinly formed on the colorless growth. No soluble pigment is observed.

[0019] (5) Tyrosine-agar medium (ISP-medium 7, cultured at 27°C)

White aerial hyphae are formed on the growth of yellowish brown [21g, Mustard Tan] to grayish yellow brown [31g Adobe Brown]. Soluble pigment of pale yellow color is observed.

(6) Nutrient agar medium (cultured at 27°C)

White aerial hyphae are thinly formed on pale yellow [2ea Lt Wheat] growth. No soluble pigment is observed.

(7) Yeast-malt agar medium (ISP-medium 2, cultured at 27°C)

White aerial hyphae are thinly formed on the pale yellowish brown [3ic Amber] growth. No soluble pigment is observed.

(8) Oatmeal agar medium (ISP-medium 3, cultured at 27 °C)

White aerial hyphae are thinly formed on the growth of colorless to pale yellow [1 1/2ca Cream]. No soluble pigment is observed.

(9) Starch agar medium (cultured at 27°C)

White aerial hyphae are thinly formed on the colorless growth. No soluble pigment is observed.

(10) Calcium malate-agar medium (cultured at 27°C)

White aerial hyphae are thinly formed on the colorless growth. No soluble pigment is observed.

[0020] 3. Physiological properties

(1) Temperature range for growth

In the tests conducted using a glucose-asparagine-agar medium (1.0% glucose, 0.05% L-asparagine, 0.05% dipotassium hydrogenphosphate, 3.0% strip agar, pH 7.0) at different temperatures of 10°C, 20°C, 24°C, 27°C, 30°C, 37°C, and 50°C, the MK299-95F4 strain grew at all temperatures tested except 10°C and 50°C. Optimum temperature for good growth appears to be in the vicinity of 27°C.

(2) Hydrolysis of starch (starch-inorganic salt agar medium, ISP-medium 4; and starch-agar medium, each cultured at 27°C) As a result of 21-day culture, hydrolysis of starch was negative in both the media.

(3) Formation of melanoid pigment (Trypton-yeast broth, ISP-medium 1; peptone-yeast-iron agar medium, ISP-medium 6; tyrosine-agar medium, ISP-medium 7; each cultured at 27°C) Formation of melanoid pigment was negative in all the media.

[0021] Utilization of various carbon sources (Pridham-Gottlieb agar medium, ISP-medium 9, cultured at 27°C)

D-glucose, D-fructose, inositol, and D-mannitol were utilizable for the growth, whereas L-arabinose, sucrose, rhamnose, raffinose lactose were not utilizable. Whether or not D-xylose was utilizable was not clear.

(5) Liquefaction of calcium malate (calcium malate agar medium, cultured at 27°C)

Liquefaction started at around the tenth day of culture and the effect was moderate.

(6) Reduction of nitrate (aqueous peptone solution containing 0.1% potassium nitrate, ISP-medium 8, cultured at 27°C)

Reduction of nitrate was negative.

[0022] In summary of the microbiological properties described above, the MK299-95F4 strain is morphologically characterized as follows: Substrate hyphae is extensively branched, giving a zigzag appearance. Fragmentation is observed. Aerial hyphae are either straight or irregularly curving, disintegrating into oval- to cylindrical-structures or spore-like structure. Neither verticillate branching nor rhizomorph nor sporangium nor motile spore is observed. White aerial hyphae are formed on the growth of colorless to pale yellow to pale yellowish brown in various media. Soluble pigment is tinged with yellow or yellowish brown in some media. Formation of melanoid pigment, hydrolysis of starch, and reduction of nitrate are all negative.

[0023] The bacterial cell components of the MK299-95F4 strain contain meso-2,6-diaminopimelic acid, arabinose, and galactose in the cell walls, exhibiting type IV cell walls. The reducing sugar in all bacterial cells was type A containing arabinose and galactose. As a result of the glycolate test, it was found to be the acetyl type. Moreover, mycolic acid was not present, phospholipid type was type PII (phosphatidylethanolamine was present; phosphatidylcholine and an unidentified phospholipid containing glucosamine were not), and main menaquinones were MK-9 (H₄).

As for fatty acid, 16:0, i-15:0, 16:1, and i-16:0 and 17:0 were the main components.

[0024] These results suggest that the MK299-95F4 strain belongs to the genus *Amycolatopsis* (literature: "International Journal of Systematic Bacteriology," 36, 29-37, 1986). Searches of the known *Amycolatopsis* strains revealed that *Amycolatopsis sulphurea* is a close relative to the MK299-95F4 strain (literature 1: id. and literature 2: "International Journal of Systematic Bacteriology," 37, 292-295, 1987). Thus, comparative analysis is being performed between the MK299-95F4 strain and the *Amycolatopsis sulphurea* strains that we have reserved. At present, the MK299-95F4 strain is identified as "*Amycolatopsis* sp. MK299-95F4". The MK299-95F4 strain was

deposited at the National Institute of Bioscience and Human-Technology under the accession number FERM P-15243 in October 17, 1995.

[0025] In implementation of the method of a second aspect of the present invention, the epoxyquinomicins A and B-producing bacterium of the genus *Amycolatopsis* is inoculated into a nutrient medium, in which the bacterium is cultured. The nutrition medium used here contains a carbon source and nitrogen source that above-mentioned producing bacterium can utilize as nutritive components.

[0026] As the nutrient sources, those typically used as nutrient sources for microorganisms, which are assimilable nutrients such as carbon sources, nitrogen sources, inorganic salts, etc., can be used. The carbon sources usable include hydrocarbons (e.g., grape sugar, malt sugar, molasses, dextrin, glycerin, and starch) and fats and oils (e.g., soybean oil and peanut oil). The nitrogen sources usable include peptone, meat extract, cotton seed powder, soybean flour, yeast extract, casein, corn steep liquor, NZ-amine, ammonium sulfate, ammonium nitrate, and ammonium chloride. The inorganic salts usable include dipotassium phosphate, sodium phosphate, salt, calcium carbonate, magnesium sulfate, manganese chloride, etc. To these amounts, trace metals, for example, cobalt, iron, etc., can be added if necessary. Further, any other known nutrient source can be used as long as it is useful for the bacteria in producing the antibiotics epoxyquinomicins A and B.

[0027] The percentage of the aforementioned nutrient sources in media is not limited; it can vary widely. The optimal composition and percentage of epoxyquinomicins A and B can easily be determined by those skilled in the art, depending on the epoxyquinomicins A and B-producing bacterium, by performing simple, small-scale experiments. Moreover, the nutrient media composed of the aforementioned nutrient sources can be sterilized in advance of culture. It is advantageous to adjust pH of the medium in the range of 6-8, particularly 6.5-7.5 before or after the sterilization.

[0028] The epoxyquinomicins A and B-producing bacteria can be cultured in such nutrient media according to the methods typically used in the production of antibiotics using common actinomycetes. Usually, culture under aerobic conditions is suitable and it can be performed while stirring and/or aerating. Culture methods that can be used include stationary culture, shaking culture, and submerged culture accompanied by aeration and stirring, but liquid culture is suitable for mass production of epoxyquinomicins A and B.

[0029] Culture temperature that can be used is not limited as long as it is in the range where the growth of the epoxyquinomicins A and B-producing bacterium is not substantially inhibited but is capable of producing the antibiotics. Culture temperature can thus be selected depending on the producing bacteria used, but temperatures in the range of 25°C to 30°C are the most preferable. Culture can usually continued until an adequate amount of epoxyquinomicins A and B has been accumulated. Although the culture duration varies with the composition of the medium, culture temperature, temperature for use, the bacterial strain to be used, etc., but typically, the target antibiotics can be obtained by culturing for 72 to 120 hours.

[0030] The amount of epoxyquinomicins A and B accumulation in medium during culture can be quantified by using *Staphylococcus aureus* Smith by the cylinder plate method, a method typically used for quantification of antibiotics.

[0031] Epoxyquinomicins A and B thus accumulated in the culture are isolated from the culture in the following manner. After removing bacterial cells from culture by the isolation methods known themselves, such as filtration and centrifugation, if necessary, following culture, the culture filtrate is adjusted to acidity (pH 2-4). The target antibiotics can be recovered through isolation and purification by using solvent extraction with organic solvents, particularly ethyl acetate, etc., adsorption and ion-exchange chromatography, or gel filtration and countercurrent distribution chromatography independently

or in combination. As carriers for adsorption and ion-exchange chromatography, activated charcoal, silica gel, porous polystyrene-divinylbenzene resin, or various kinds of ion exchange resin can be used. In addition, from the isolated bacterial cells, target antibiotics can be extracted, and isolated and purified by solvent extraction using a suitable organic solvent or elution method by bacterial cell disruption in the same manner described above. The novel antibiotics epoxyquinomicins A and B with the above-described properties are thus obtained.

[0032] Further, in a third aspect of the present invention, there is provided an antibacterial agent that contains epoxyquinomicins A and (or) epoxyquinomicin B, represented by the general formula (I), or their pharmaceutically acceptable salt(s) as active ingredient(s).

[0033] Further, in a fourth aspect of the present invention, there is provided an antitumor agent that contains epoxyquinomicins A and (or) epoxyquinomicin B, represented by the general formula (I), or their pharmaceutically acceptable salt(s) as active ingredient(s).

[0034] In this antibacterial agent or antitumor agent, epoxyquinomicins A and (or) B or their (its) salt(s) as active ingredient(s) can be a composition mixed with pharmaceutically-acceptable solid or a fluid carrier in ordinary use, such as ethanol, water, starch, etc.

[0035] Further, in the fifth aspect of the present invention, there is provided *Amycolatopsis* sp. MK299-95F4, which has the property of producing the antibiotics epoxyquinomicin A and epoxyquinomicin B represented by the aforementioned general formula (I).

[0036]

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention will be more specifically described by giving examples hereinbelow. However, the scope of the present invention is not limited to these examples.

[0037]

EXAMPLE 1

Production of the antibiotics epoxyquinomicins A and B

A liquid medium (pH 7.0) containing 0.5% glycerin, 2% sucrose, 1% soybean flour, 1% dry yeast, 0.5% corn steep liquor, and 0.001% cobalt chloride was placed (110 ml each) in 500 ml Erlenmeyer flasks, and sterilized at 120°C for 20 min by the conventional method. Subsequently, *Amycolatopsis* sp. MK299-95F4 (FERM P-15243) was taken from its agar slant culture and then inoculated into the aforementioned medium, followed by shaking culture at 30°C for 5 days to obtain a seed culture.

[0038] A liquid medium (pH 7.4) containing 2% glycerin, 2% dextrin, 1% Bacto Soyton, 0.3% powder yeast extract, 0.2% ammonium sulfates, 0.2% calcium carbonate, and one drop of silicone oil was placed (110 ml each) in 500 ml Erlenmeyer flasks, and sterilized at 120°C for 20 min by the conventional method. 2 ml portions of the aforementioned seed culture were inoculated into these media, followed by shaking culture at 27°C for 4 days.

[0039] The resulting culture was filtered to isolate bacterial cells. 2.55 l of the recovered culture filtrate was adjusted to pH 2 with 6N-HCl and then extracted by an equal volume of butyl acetate. The butyl acetate layer was dried with anhydrous sodium sulfate. The butyl acetate was concentrated to dryness under reduced pressure. The residue was dissolved in 50 ml of methanol, washed twice with 5 ml of hexane, and the methanol layer was concentrate to dryness under reduced pressure.

[0040] The resulting residue was separated by chloroform-methanol-water (50:10:40, 100 ml) and the lower layer was concentrated to dryness under reduced pressure to yield a brown oily substance (0.515 g). This oily substance was subjected to silica gel column chromatography (Kieselgel 60, Merck Co., 50 ml) and sequentially eluted with a toluene-acetone mixed solvent (10:1, 7:1, 5:1, 3:1, and 2:1). The resulting active fractions were subjected to silica gel column chromatography under the same conditions and

sequentially eluted with a toluene-acetone mixed solvent (50:1, 20:1, 10:1, and 7:1) to afford a 124 mg mixture containing epoxyquinomicins A and B. 35 mg of this mixture was isolated and purified by silica gel TLC (developing solvent: chloroform-methanol, 20:1).

[0041] Epoxyquinomicin A was obtained as a 20 mg light yellow powder with a melting point of 168-173°C (degradation) and epoxyquinomicin B as a 10 mg light yellow powder with a melting point of 178-184°C (degradation).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows an ultraviolet absorption spectrum of epoxyquinomicin A in methanol solution.

FIG. 2 shows an ultraviolet absorption spectrum of epoxyquinomicin A in 0.01N NaOH-methanol solution.

FIG. 3 shows an ultraviolet absorption spectrum of epoxyquinomicin A in 0.01N HCl-methanol solution.

FIG. 4 shows an infrared absorption spectrum of epoxyquinomicin A measured by the KBr tablet method.

FIG. 5 shows a proton nuclear magnetic resonance spectrum of epoxyquinomicin A measured with heavy methanol solution (internal standard: trimethyl silane).

FIG. 6 shows a carbon-13 nuclear magnetic resonance spectrum of epoxyquinomicin A measured with heavy methanol solution (internal standard: trimethyl silane).

FIG. 7 shows an ultraviolet absorption spectrum of epoxyquinomicin B in methanol solution.

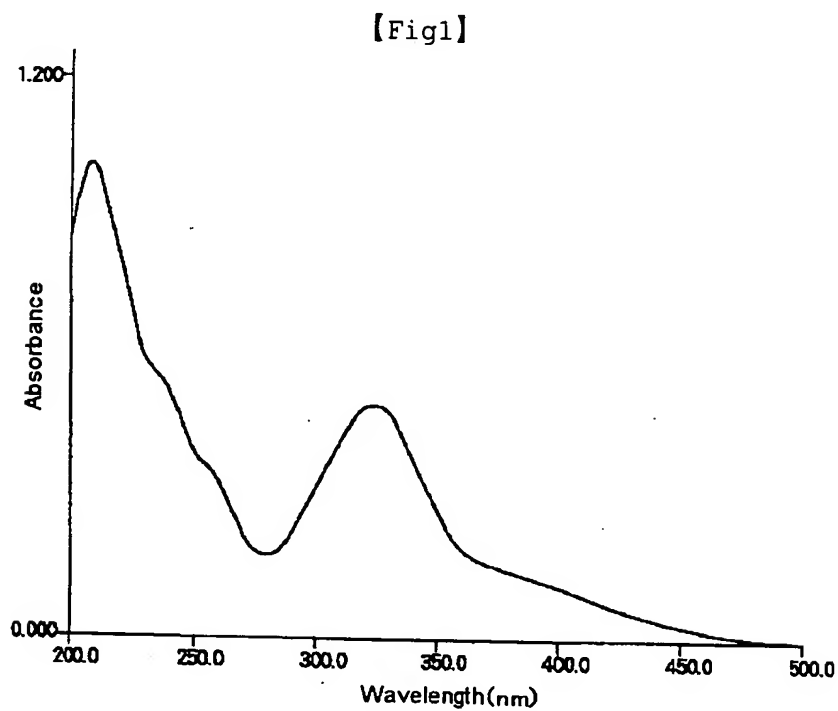
FIG. 8 shows an ultraviolet absorption spectrum of epoxyquinomicin B in 0.01N NaOH-methanol solution.

FIG. 9 shows an ultraviolet absorption spectrum of epoxyquinomicin B in 0.01N HCl-methanol solution.

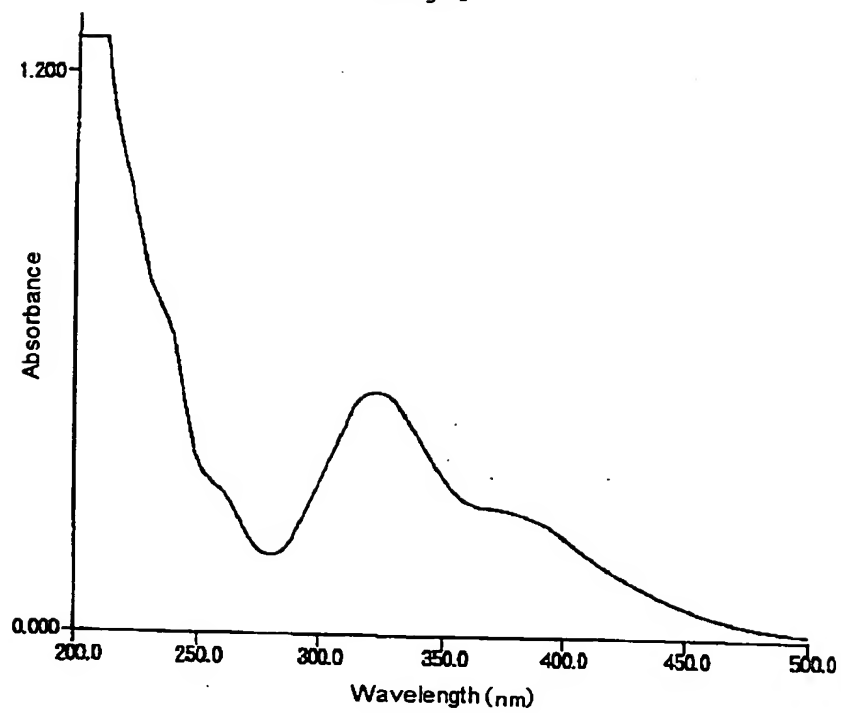
FIG. 10 shows an infrared absorption spectrum of epoxyquinomicin B measured by the KBr tablet method.

FIG. 11 shows a proton nuclear magnetic resonance spectrum of epoxyquinomicin B measured with heavy methanol solution (internal standard: trimethyl silane).

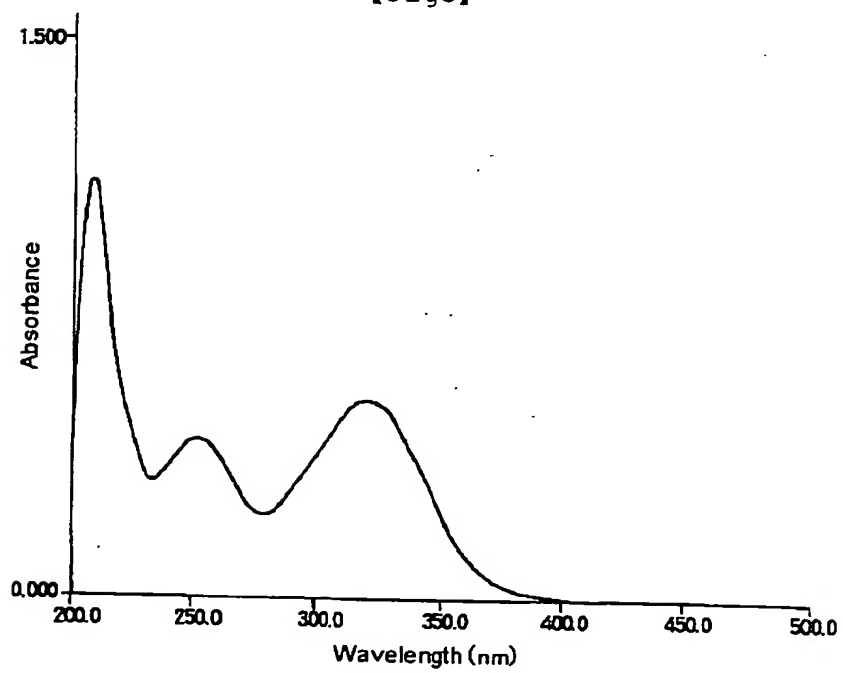
FIG. 12 shows a carbon-13 nuclear magnetic resonance spectrum of epoxyquinomicin B measured with heavy methanol solution (internal standard: trimethyl silane).



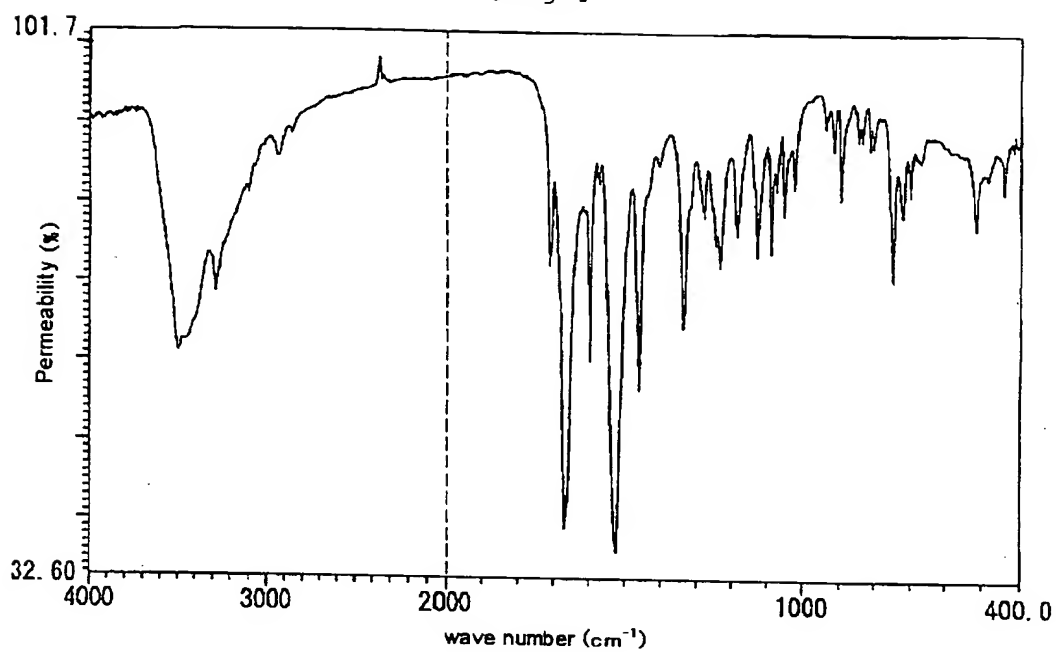
【Fig2】



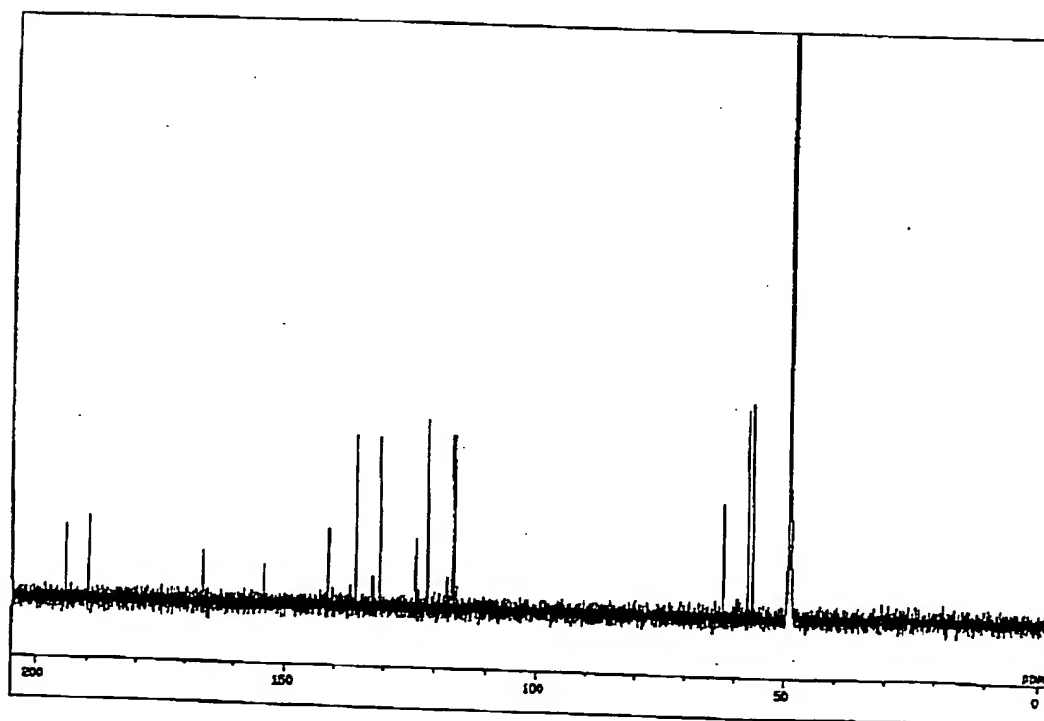
【Fig3】



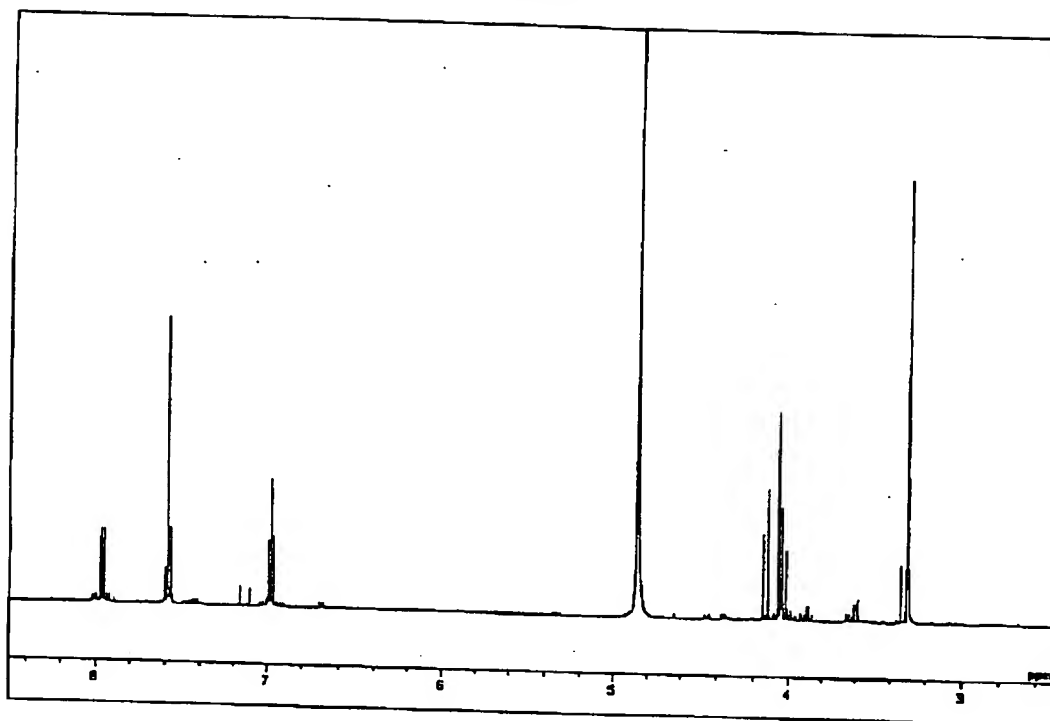
【Fig4】



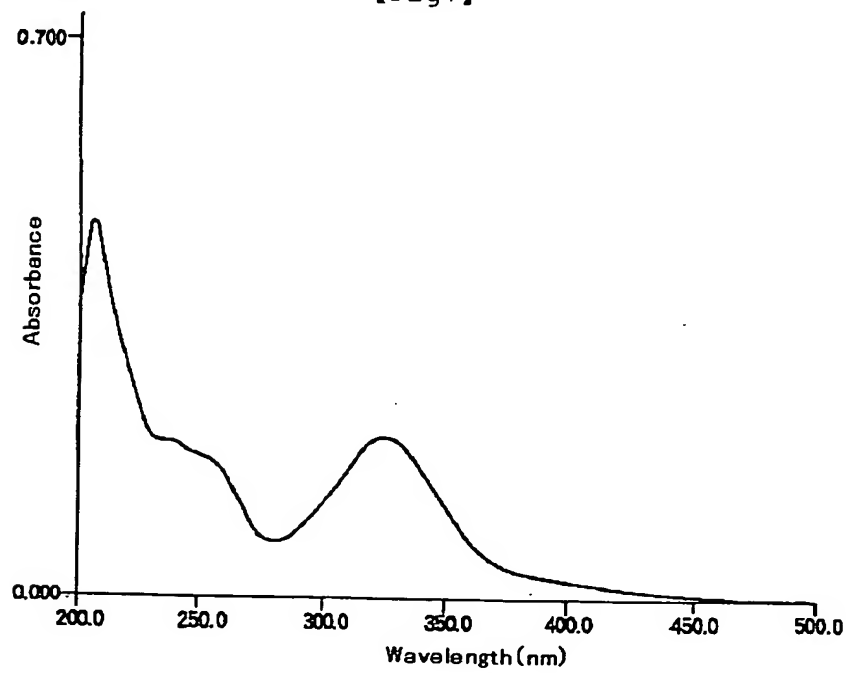
【Fig5】



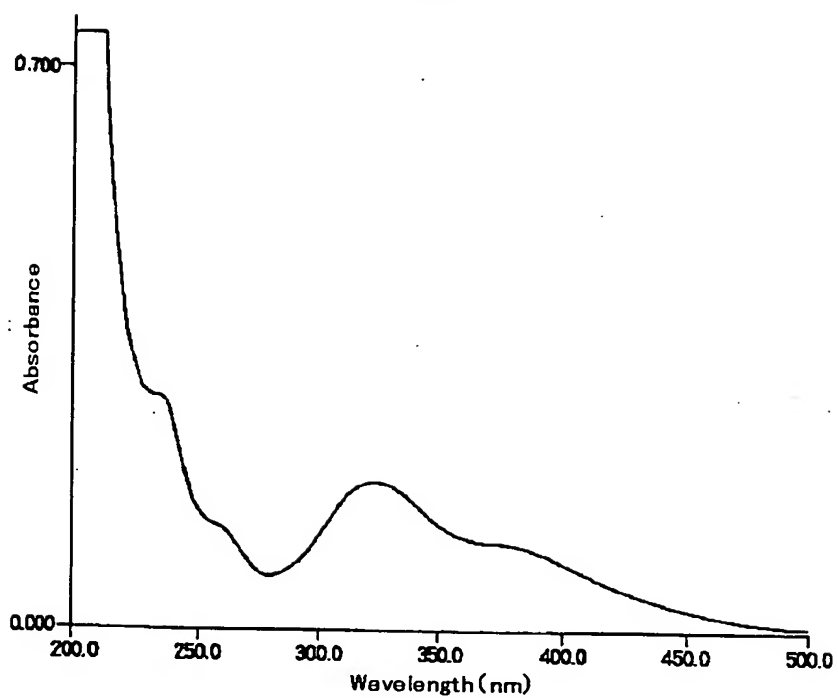
【Fig6】



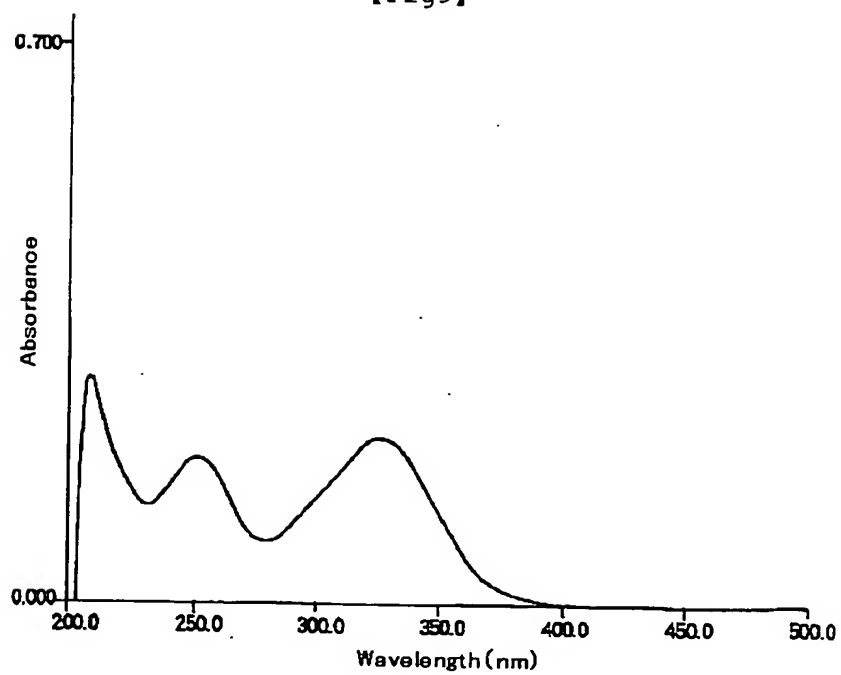
【Fig7】



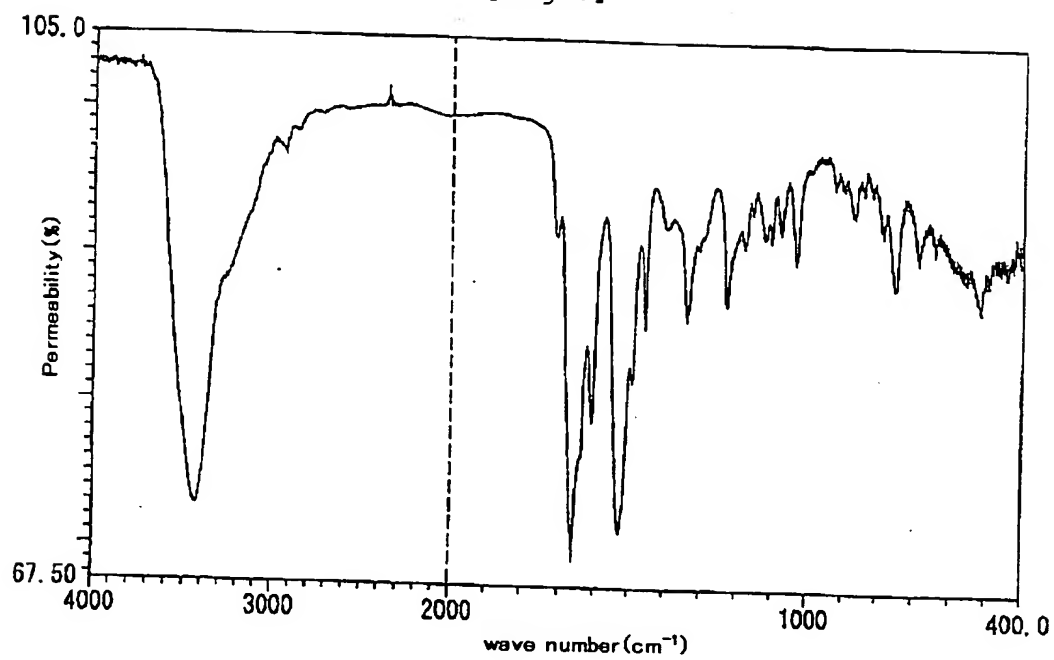
【Fig8】



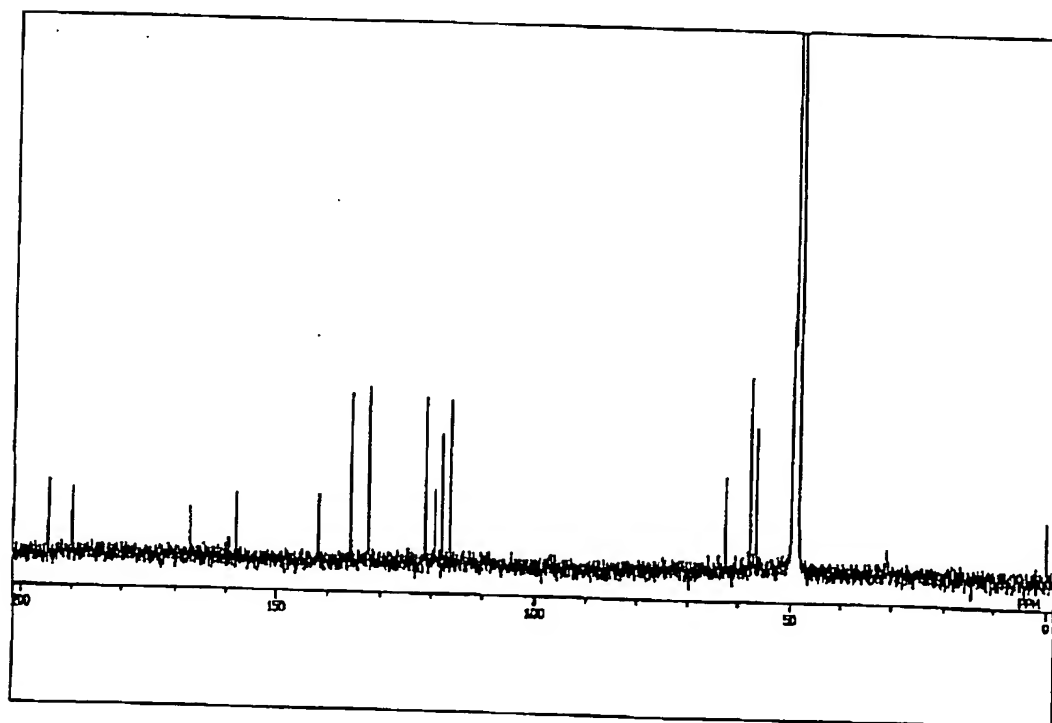
【Fig9】



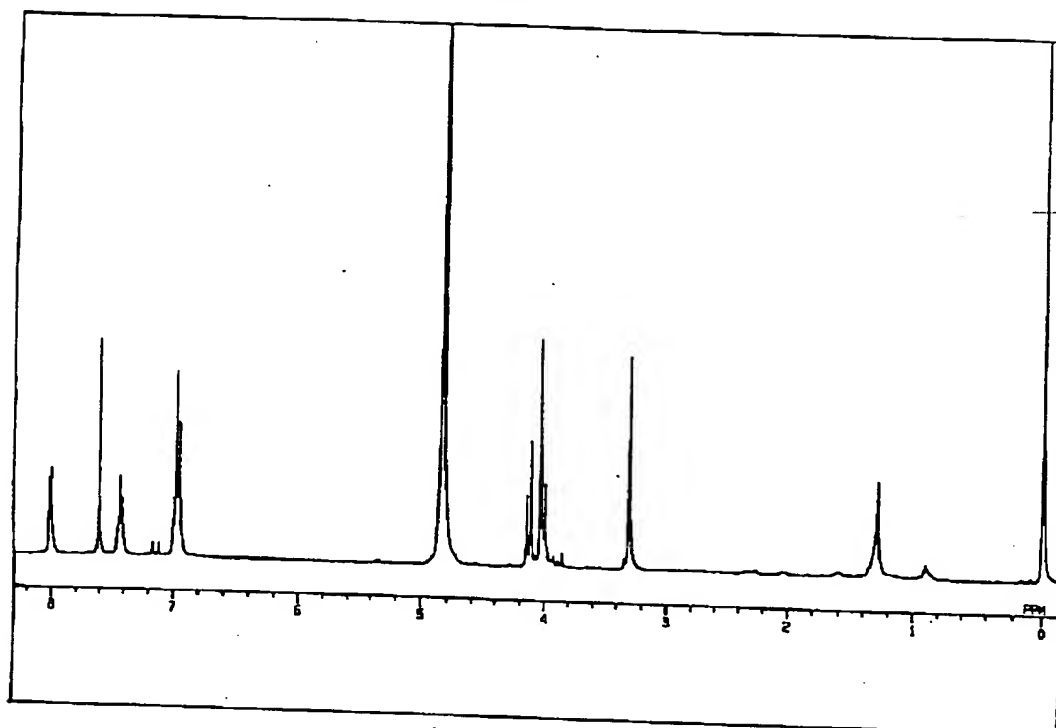
【Fig10】



【Fig11】



【Fig12】



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